STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR FORMALIN USE AS A FUNGICIDE UNDER INAD #9013

Sponsor:

U.S. Fish and Wildlife Service, Division of Fish Hatcheries

_____ _____
Sponsor Signature Date Approved

Suppliers:

Argent Chemical Laboratories
Natchez Animal Supply Company
Western Chemical Inc.

Facility for Coordination of Formalin (Fungicide) INAD:

Bozeman National INAD Office
4050 Bridger Canyon Road
Bozeman, Mt 59715

Proposed Starting Date	October 14, 1995
Proposed Ending Date	October 13, 1996
Study Director	Mr. Jim Bowker

_____ _____
Study Director Signature Date

Clinical Field Trial Location and Trial Number:

_____ _____
Type or Print Facility Name Trial Number

Investigator_____
Type or Print Name

_____ _____
Investigator Signature Date

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR FORMALIN USE AS A FUNGICIDE UNDER INAD #9013

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of formalin to control fungal infections on a variety of fish species and their eggs. INAD #9013.

II. SPONSOR

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715. Phone: 406/587-9265 ext. 125; FAX: 406/582-0242; Email: dave_erdahl@fws.gov

Suppliers: Argent Chemical Laboratories, 8702 152nd Ave. N.E., Redmond, WA 98052. Telephone: 800/426-6258

Natchez Animal Supply Co., 201 John R. Junkin Dr., Natchez, MS 39120. Telephone: 800/647-6760

Western Chemical Inc., 1269 Lattimore Road, Ferndale, WA 98248. Telephone: 206/384-5898

Study Director: Mr. Jim Bowker, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715. Phone: 406/587-9265 ext. 126; FAX: 406/582-0242; Email: jim_bowker@fws.gov

Principal Regional INAD Coordinators: See Appendix I for names and addresses.

Study Monitors for Formalin (Fungicide) INAD: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses. Each facility has been assigned a trial number that reflects the INAD number (9013) and a unique number for that facility (e.g., Abernathy STC 9013 - 01).

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date: October 14, 1995

Proposed Completion Date: October 13, 1996

V. BACKGROUND/PURPOSE

A. Background:

Every major cultured aquatic species is plagued with fungal infection problems. Affected species include finfish (freshwater and marine), oysters, clams, crabs, penaeid shrimp, crayfish, and lobster (Schnick 1984, Schiewe et al. 1988, Sindermann 1988 a, b, c, Fisher 1988, Lightner 1988, Beleau and Plumb 1987, Harrell 1987, Busch 1987, Thune 1987, Bell and Lightner 1987, McVicar and McLay 1985, Langvad et al. 1985). Fungal infections cause substantial monetary losses to aquaculturists (Bailey and Jeffrey 1989, Pickering and Willoughby 1982, Langvad et al. 1985, McVicar and McLay 1985).

The organisms responsible for major fungal infections on fish are, for the most part, Saprolegniaceae of three genera--*Saprolegnia*, *Achlya* and *Dictyuchus*. Penaeid shrimp have problems with *Lagenidium*. These organisms are highly opportunistic and generally cause cutaneous infections so bath treatments are effective (Neish and Hughes 1980, Pickering and Willoughby 1982, Alderman 1982, Post 1987).

Dead fish eggs act as growth media for fungi that can then be responsible for killing living fish eggs by suffocation and invasion. Fishes, on the other hand, are susceptible to fungal infections as a secondary invasion from injuries and stress or presence of dead fish that are reservoirs for production of zoospores of *Saprolegnia* (Post 1987).

Fungal infections on fish eggs, if not treated, can cause major losses and, thus, affect the restoration and preservation of depleted stocks of fish cultured by the U.S. Fish and Wildlife Service (USFWS). The extent of losses of fish from saprolegniasis depends upon the severity of the primary cause of fungal infection. Morbidity can vary from less than 10% to total loss of the population (Post 1987).

Since 1933, the worldwide treatment of choice from most aquatic fungal infections has been malachite green. A replacement was needed because malachite green never has been registered by the U.S. Food and Drug Administration (FDA) for fishery use. In addition, abnormalities in development have occurred in the young of several animal species exposed to high concentrations and it is considered to be potentially hazardous to humans (Meyer and Jorgenson 1983, Schnick 1989a).

Efforts to register drugs and chemicals for aquaculture (in the U.S.) have centered on finding a replacement for malachite green primarily as a fungicide and, secondly, as a protozoacide. The Aquaculture Work Group of the U.S. Department of Agriculture Interregional Research Project No. (IR-4) and FDA identified a replacement for malachite green as its number one priority need. All aquaculture groups at IR-4/FDA Workshops have endorsed the selection of that priority (Schnick 1984, 1987, 1989 b and c). The Task Force on Therapeutic Compounds of the Joint Subcommittee on Aquaculture (1988) developed a set of criteria to establish priorities of need and identified a replacement for malachite green as a high priority for funding.

A worldwide search to find a replacement for malachite green has been underway since the early 1980's (Alderman 1985, 1982). Although many compounds are effective on fungus *in vitro*, they will either not be effective *in vivo* or are toxic to fish or fish eggs. Over 200 compounds have been tested in the

U.S. alone (Meyer and Schnick 1989; Bailey 1984; Bailey and Jeffrey 1989).

The La Crosse National Fisheries Research Center (NFLX) has screened several hundred compounds to find a replacement for malachite green as a fungicide. In the process, NFLX has found 1667 mg/L of formalin to be very effective in reducing the infection rate and improving the hatch rate of eggs. In fact, formalin produced a better hatch rate than any of the candidate antifungal agents or malachite green (Schreck et al. 1992). Information on the control of fungal infections with formalin on the fish themselves is not as well documented; however, fish hatchery managers and fish health professionals in the Pacific Northwest found formalin to be effective in controlling fungal infections of Pacific salmon if the infections had not progressed too far.

In 1986, the FDA approved a new animal drug application (NADA) for the use of formalin to control external parasites (*Icthyopthirius, Chilodonella, Costia, Scyphidia, Epistylis, Trichodina, Cleidodiscus, Gyrodactylus, and Dactylogyrus*) on several fish species (salmonids, catfish, largemouth bass, and bluegill) and to control fungal infections on the eggs of salmon, trout and esocids. This decision by FDA was based on data that illustrated formalin was effective against those disease organisms and safe to use on those species allowed on the label. Other cultured fish species could benefit from the use of formalin as a parasiticide and fungicide, both on the eggs and the fish themselves.

D. Purpose of INAD:

This compassionate investigational new animal drug (INAD) request is for the extension of the formalin label for control of fungal infections on species of fish and/or eggs that are not covered by the current NADA and are cultured by the U.S. Fish and Wildlife Service (USFWS). The purpose of this compassionate INAD on formalin is to develop clinical field trial data that will be used to determine the most appropriate treatment regime for controlling fungal infections in a variety of cultured fish and eggs. These data will be used to support an extension of the NADA for formalin.

USFWS anticipates requesting the FDA to grant extensions of the formalin INAD for additional years at the end of the first treatment season. The USFWS is aware that disease outbreaks requiring formalin as a fungicide therapy are unpredictable. There is no way of knowing in advance if, when, or where opportunities for pivotal studies will be encountered. The USFWS feels that data from at least three treatment seasons will be required in order to adequately assess the efficacy of formalin for treatment of fungal infections on certain fish species and fish eggs to support an extension of the NADA for formalin.

VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the efficacy of formalin under typical hatchery situations for treatment of fungal infections on fishes and eggs of fish species that are not currently covered by the formalin NADA.

2. Provide the opportunity for fish culturists to legally use formalin to control fungal infections and maintain healthy fish stocks during the time necessary for collection of efficacy, safety, and residue data required for an extension of the NADA on formalin.

VII. MATERIALS

A. Test and control articles:

1. Drug Identity

a. Active ingredient

Trade Name: **Parasite-S (NADA 140-989; Formalin-F (NADA 137-687); Paracide-F (NADA 140-831)**

Chemical Name: Formalin (Formaldehyde solution)

Chem Abstr Reg No: 50-0-0

Appearance: Colorless liquid

Molecular Formula: CH_2O

b. Strength and dosage form

Formaldehyde solution is approximately 37% by weight of formaldehyde gas in water, with 10-15% methanol added as a stabilizer.

c. Manufacturer, source of supply

Argent Chemical Laboratories
8702 152nd Ave. N.E.
Redmond, WA 98052
Telephone: 800/426-6258

Natchez Animal Supply Co.
201 John R. Junkin Dr.
Natchez, MS 39120
Telephone: 800/647-6760

Western Chemical Inc.
1269 Lattimore Road
Ferndale, WA 98248
Telephone: 206/384-5898

The shipment procedure for formalin is as follows: Argent Chemical Laboratories, Natchez Animal Supply Co., or Western Chemical Inc. to Investigators (See Section VII.A.6 Accountability [page 6] for details and Appendix IIIa for names and addresses of Investigators).

2. Verification of drug integrity/strength:

The suppliers will provide the analytical data necessary to establish purity of each lot of formalin supplied. The lot number and date of manufacture for each batch of formalin will be placed on the label of each container. The form "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (Form 1) will clearly identify the lot number and date of manufacture of formalin shipments. If the integrity of the formalin is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded, dated, and signed in the Chemical Use

Log (Form 2). The Study Monitor assigned to the Investigator involved will be immediately notified and the remaining material will be returned to the Study Monitor along with the properly recorded Form 1.

3. Storage Conditions

Formalin will be stored in the original container provided by the supplier with the appropriate investigational label attached. Formalin drums are to be stored at or above room temperature (20°C). Formalin should not be stored at temperatures much below 20°C because paraformaldehyde, a white precipitate, may form at the bottom or on the wall of the container.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for formalin (Appendix IV). Each person involved with the study and each person who may be present during the use of formalin shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with formalin. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., will be worn at all times.

5. Investigational labeling

Copies of the labels to be attached to each container of formalin are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of formalin.

6. Accountability

Each USFWS Investigator will notify FDA prior to any shipment of formalin for use under this INAD. Immediately upon placing an order with the approved supplier, the Investigator will complete Form 1, "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" and send it to his/her Study Monitor. The Study Monitor will then send the original plus two copies to the FDA. Both the Investigator and the Study Monitor are required to sign Form 1. The Study Monitor will also send a single copy of Form 1 to the Study Director at the Bozeman National INAD Office. The Investigator will keep one copy of the completed Form 1 for the facility's INAD file. Arrangements should be made between Investigators and Study Monitors to insure completed Form 1s are received by the FDA within 7 days of the date an order was placed.

Investigators are also responsible for maintaining an accurate inventory of formalin on-hand. A Chemical Use Log (Form 2) will be supplied to each Investigator. Each time formalin is used, it must be reported by the Investigator on Form 2.

At the conclusion of the study, the Study Monitor will verify the quantity of formalin remaining against the Chemical Use Log (Form 2). All remaining formalin will then be available for other legal use at the station following removal of the INAD label.

7. Preparation Procedures

The amount (volume) of formalin needed for each treatment will be volumetrically measured and uniformly mixed in the holding water to achieve the prescribed treatment concentration (bath) or metered for a calculated time at a flow rate adequate to achieve the desired treatment concentration (flowing system).

B. Items Needed for Sample Collection, Observations, Etc.:

Sampling and diagnostic equipment should include scissors, forceps, clean microscope slides and cover slips, 90% methyl alcohol, methylene blue, Bouins solution, dextrose agar, peptone-dextrose agar, inoculation loops, Bunsen burner, and a compound microscope. This equipment may be supplied by the Study Monitor.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the formalin (fungicide) INAD will need to complete several forms. These forms are described in Section XIII (p 13). Copies of these forms are attached to this Study Protocol.

VIII. EXPERIMENTAL UNIT

The experimental unit in this clinical field trial will consist of a contained or isolated group of fish or eggs. This will generally be a group of fish contained in a tank, raceway, or pond, or lot of eggs held in an incubator. The experimental unit will **not** be individual animals or eggs.

IX. ENTRANCE CRITERIA

A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of this Study Protocol before formalin can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, by an Investigator, or by a Study Monitor to control emergency disease outbreaks (See Section XX).

B. The characteristics of the study animals (species, size, number, etc.) is presented in Appendix VI.

C. Diagnosis of disease

1. Saprolegniasis

a. Saprolegniasis is a fungal disease of fish and fish eggs caused by a member of the family Saprolegniaceae. This name is broadly accepted when the etiology is a species of the genus *Saprolegnia, Achlya, or Dictyuchus*. The presumptive diagnosis will involve observation of fish or egg appearance. Most diagnoses will be performed by the Investigator or confirmed by the Study Monitor if questions arise. The appearance of a fluffy, cotton-like, white to gray-white or gray-brown growth on the skin, fins, gills, or eyes of fish or fish eggs is a presumptive positive diagnosis for saprolegniasis. The usual practice is to accept the presumptive diagnosis and initiate therapy (Post 1987).

b. Definitive diagnosis of saprolegniasis is based upon identification of the fungi. A small mycelial sample is taken from the infected fish or egg tissue, mounted on a microscope slide with several drops of water, and examined. Fungi causing saprolegniasis have branched, nonseptate hyphae. These organisms have zoosporangia at the tip of fertile hyphae for asexual reproduction. Mature primary zoospores have two flagella; encystment occurs, and secondary zoospores also with flagella are produced by all *Saprolegnia sp. Achlya sp.* do not produce secondary zoospores, and *Dictyuchus* sp release only secondary zoospores (Post 1987).

c. The presence of fungal infections in early stages of development can be detected without handling the fish by using a procedure developed by Dr. Larisa Ford at the National Fish Health Research Laboratory at Leetown, West Virginia. The procedure involves the identification of fungal growth on bacteriological media from filtered water samples (Personal communication, John Coll, U.S. Fish and Wildlife Service, Lamar, Pennsylvania).

2. Branchiomycosis

a. Branchiomycosis is a fungal disease of fish gill tissue. Presumptive diagnosis will involve observation of fish appearance and behavior. Most diagnoses will be performed by the Investigator or confirmed by the Study Monitor if questions arise. Fishes with acute to subacute branchiomycosis may appear to be weak and lethargic. They may exhibit respiratory distress and an intolerance to handling. Fungus develops on or in gill tissue and gills may appear bright red from impaired circulation. Some gill areas may be white to brown depending on the stage of necrosis. Gills may become ragged and corroded in appearance. No disease signs may be evident during chronic cases. Pale areas on gills may be present and some lamellae may appear swollen with fungal thrombi and slight to moderate necrosis. Diagnosis of branchiomycosis requires three essential findings: (1) history of disease presence in the geographic area, (2) disease signs as previously stated, and (3) detection and identification of the fungus in gill tissue. Pieces of gill arch mounted in water on microscope slides may reveal gill necrosis. Squash preparations examined may reveal fungal hyphae and spores. Sections cut and stained from infected gills should contain hyphae, syncytia and spores. The previously mentioned disease signs and presence of fungus in gill tissue are presumptively positive for branchiomycosis (Post 1987).

b. Definitive diagnosis of branchiomycosis requires that measurements be made of isolated hyphae and spores from either cut and stained sections of infected gills (Sindermann and Rosenfield 1954) or from hyphae and spores cultured on Sabourand's dextrose agar or peptone-dextrose agar at pH 5.8 and 25 to 30°C (Meyer and Robinson 1973).

D. Level of disease

The level of fungal infection should be low or in the early stages of development to obtain control by formalin treatments. Therefore, prompt diagnosis and treatment is imperative. Fungal infections undetected and untreated until the advanced stages may result in mortalities even with treatment. Fish should be treated by the prescribed regimes as soon as disease signs are evident or a positive presumptive diagnosis is made. Treatment of fish eggs should typically begin 24 hours after fertilization and continue through hatch according to prescribed regimes. Treatment will not be mandatory and will be at the discretion of the Investigator.

E. Prophylactic Treatment

Prophylactic (preventative) treatment of fish with formalin is allowed under this INAD exemption. However, prophylactic treatment should be administered **only** when station/facility case history records indicate that the potential for fungal infection is elevated. Past culture experience may indicate that when a certain species/strain of fish (of a particular size) are reared at a specific density or flow index, or are reared under specific water quality/environmental parameters at a certain time of the year, disease is very likely to result. Under such conditions,

prophylactic treatment with formalin is justified.

F. Environmental conditions

See Appendix VII for details on environmental considerations at each facility.

G. Ability of investigator to fulfill all the requirements of the Study Protocol

See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

X. TREATMENT GROUPS

A. A treatment group or experimental unit will generally be the entire tank, pond, raceway, or egg incubator that becomes infected.

B. Control groups will not be a requirement for clinical field trials evaluating the efficacy of formalin treatment due to the following:

1. Outbreaks of fungal infection often occur in only one tank or raceway at a time.

2. Fungal infections may result in complete mortality in untreated controls. Such a risk cannot be taken when fish or egg stocks are valuable or limited (e.g. threatened/endangered species).

3. Separating diseased fish into control and treatment groups may not only increase the stress placed on fish, but may also change environmental conditions such as population density, water quality, etc. These factors may impact the rate of progression of fungal infection. Although it may be possible to minimize such bias by transferring two sub-groups of "sick" fish into two separate, but equal tanks (where one group will receive treatment and the second will serve as a non-treated contro), such "design" is not an option at many facilities. Furthermore, as diseased fish are reservoirs of fungi, whenever fish are transferred to new rearing units the potential for infection may be increased.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. Control groups are extremely important to not only document disease virulence and disease response to treatment, but also to validate potential adverse reactions in treated animals. Assignment to control and treatment groups should be random and designed to avoid bias. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Use of control groups will ensure that results of efficacy studies provide useful information that will support a NADA.

Many facilities participating in this INAD are doing so because previous experience has indicated higher survival among formalin treated fish. In situations where some fish losses can be accepted, controlled tests should be conducted. However, as stated above, it is important that all fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Separating diseased or stressed fish into new groups for treatment may change environmental conditions responsible for the disease in the first place, thereby rendering formalin therapy meaningless.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which mortalities are tallied and morbidity observations recorded by individuals who are unaware which fish have been treated and which fish are controls.

XI. TREATMENT SCHEDULES

A. Route of administration

Formalin will be administered to eggs by delivering a metered flow of formalin into the egg holding containers at a flow rate adequate to obtain the prescribed treatment concentration for the prescribed duration. Fish will be treated by either a static bath or flow-through treatment. Static, low-level treatments for indefinite or long-term periods may be used in some cases. For the most common treatments, formalin is mixed directly with the holding water and applied as an immersion bath at a specified concentration and duration and then flushed from the holding water, or metered to the holding water at a flow rate adequate to achieve the desired treatment concentration for a specified duration in a flowing system.

B. Dose to be administered

1. Treatment of disease

Formalin may be applied as an immersion bath or flow-through treatment at concentrations ranging from 15 - 2000 mg/L. Within this range, the concentration applied will be at the discretion of the Investigator.

2. Prophlactic treatment

Formalin may be applied as an immersion bath or flow-through treatment at concentrations ranging from 15 - 2000 mg/L. Within this range, the concentration applied will be at the discretion of the Investigator.

C. Dosing interval and repetition

1. Treatment of disease

The recommended dosing interval will be at the discretion of the investigator. In the case of eggs, formalin treatment will generally be administered daily or every other day until hatch. In the case of fish, formalin treatment will generally be administered every other day to weekly until the fungal infection has been controlled.

2. Prophylactic treatment

The recommended dosing interval will be at the discretion of the investigator. In the case of eggs, formalin treatment will generally be administered daily or every other day until hatch. In the case of fish, formalin treatment will generally be administered every other day to weekly until the fungal infection has been controlled.

D. Duration of treatment

The duration of treatments will be at the discretion of the investigator. Egg treatments will generally last for 15 minutes, although in some cases may extend longer. Fish treatments will generally last for 30-60 minutes, although in certain

situations they may also extend longer (e.g. treatment at 15 mg/L may last an indefinite period). After completion of treatment, either the treatment solution should be flushed from the rearing unit or the fish should be removed to fresh water.

E. Detailed procedures for drug administration

Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc. should be worn at all times when working with formalin. Formalin will be volumetrically measured for each treatment and each use documented on Form 2. Respirators specially equipped with formalin cartridges will be used by applicators if any irritation is noted from formalin vapors or if the concentration of formalin in the air reaches the 0.5-ppm action level for an 8-hour, time-weighted average or 2.0 ppm for a 15-minute exposure, as per the Code of Federal Regulations (29CFR 910.1048). Personnel using respirators will be provided with the appropriate training for use and maintenance. An up-to-date copy of the MSDS will be kept on hand at each participating facility. Participating personnel will be required to be familiar with and follow the MSDS guidelines.

To aid in the uniform distribution of chemical, formalin should be solublized in a water stock solution prior to treatment. The stock solution should be administered to the fish in either of two ways:

1. The stock solution should be mixed into a static system (i.e., bath) to achieve the desired concentration. After treatment is completed, either the fish should be removed to fresh water or the chemical should be flushed from the system.

2. The stock solution should be administered to a flowing system using a metering system or drip station. The stock solution should be added at a flow rate adequate to achieve the desired treatment concentration. The volume of stock solution should be sufficient to maintain a constant inflow of formalin for the desired treatment duration..

F. Permissible concomitant therapy

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other treatments once a decision has been made to conduct formalin therapy. However, if concomitant therapy is required in order to protect valuable fish stocks, it should be fully documented and the efficacy data from the formalin treatment involved should be appropriately labeled.

If after a treatment major mortalities are occurring and the causative agent is still present, the Study Monitor should be consulted.

XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins either with the detection of a disease condition warranting formalin treatment or with the decision to treat valuable fish based on hatchery records indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form 3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

Morbidity and mortality data, coupled with case history and gross observation of fish and/or eggs, usually indicate when formalin treatment is needed. If treatment is for an identified disease condition, **source data must be collected for at least 10 days before treatment, during treatment, and for at least 14 days after the last treatment**. If treatment is initiated for the prevention or mitigation of a potential disease condition, **source data should indicate fish health status prior to treatment, as well as morbidity/mortality during treatment and for at least 14 days following treatment, or until the suspected period of increased disease risk has passed**. If the period of suspected disease risk lasts for a considerable period of time, the Investigator may choose to record mortality only on days that mortality actually occurs (to save space on Form 3). Collection of this data is critically important in all cases. Post-mortem examinations should be performed periodically on a representative sample of fish to establish that the cause of death is in fact from fungal infection.

As a result of the potential diversity of treatment circumstances involved in these studies, Investigators are encouraged to provide copies of their own daily mortality record forms for individual rearing units. Investigators may also choose to create their own forms for purposes of recording source data under this INAD. **Supplementary data forms should be attached to Form 3.**

2. Secondary Parameters

Secondary parameters include general observations on fish behavior and response to routine culture activities. Secondary parameters would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

3. Adverse Reactions

Any adverse reaction to treatment should be reported immediately to the Study Monitor, who will in turn notify the Study Director. Such responses might include changes in water quality, extremely negative responses/behavior by the fish, or hazards to the applicator. Although formalin has been used fairly extensively with beneficial effect, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions. If any signs of drug toxicity are detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.

Note: Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

XIII. FORMS FOR DATA COLLECTION

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the formalin as a fungicide INAD will need to complete the following forms:

Form 1. Guide for reporting investigational new animal drug shipments for poikilothermal food animals.

Form 2. Chemical use log for clinical field trials using formalin (fungicide) under INAD #9013.

Form 3. Diagnosis, treatment, and mortality record for clinical field trials using formalin (fungicide) under INAD #9013.

Form 4. Disposal record for animals from clinical field trials using formalin (fungicide) under INAD #9013.

Copies of these forms are attached to this Study Protocol.

XIV. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data should be original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it. Each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes. The original record should be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during treatment should be disposed of by burial or incineration. Treated fish will be maintained at culture facilities for at least 5 days following final treatment before they are stocked or allowed to enter the food chain.

No withdrawal period will be required for fish that will not be catchable for 5 or more days after release or are illegal for harvest during that 5 day period. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

The Investigator must record the disposition of all treated fish on Form 4.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

Formalin will be used only in the manner and by the individuals specified in the Study Protocol. The investigational drug may not be redistributed to others not listed in the Study Protocol. At the conclusion of the study, all remaining formalin should be retained at the facility. The Study Monitor will then verify the quantity of formalin remaining against the Chemical Use Log. All remaining formalin can then be used for other legal uses.

XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES

A. Drug distribution

See Section VII.A.6. Accountability (page 6) for information and details.

B. Study Monitors

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. There is one Study Monitor assigned to each facility within the USFWS that is covered by the formalin (fungicide) INAD. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. The Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

C. Special equipment and materials

Most of the equipment and materials required for this study (with the exception of the formalin itself) are already available at each participating fish hatchery. Diagnosis and treatment of diseases of fish is a common occurrence at most fish hatcheries. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 6).

D. Administrator of the drug

Formalin will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Formalin will be maintained in a secure location, and only the Investigator or a person under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 6) for details and Forms 1-4 for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitors who will ensure that all required information is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare an annual report that will be submitted to the FDA.

G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data (with the exception of Form 1, in which case the original is forwarded to FDA through the Study Monitor, See Section VII.A.6. Accountability page 6 for complete details). Original raw data on Forms 2 and 4 will be retained by the Investigator until completion of the study, at which time copies will be sent to the Study Monitors. Copies of Form 3 will be sent to the Study Monitors on a quarterly basis. The Study Monitors will carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor will contact the Investigator immediately to rectify the problem. After review, Study Monitors will forward all data to the Study Director. As stated above, the complete set of raw data will be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the Bozeman National INAD Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared for submission to the Sponsor who will in turn submit the report to the FDA. This submission will probably include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XIX. PROTOCOL AND PROTOCOL AMENDMENTS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before a study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative). Copies of the signed amendment will be attached to each copy of the Study Protocol. Investigators will be liable for non-compliance violation if drugs are used without a Study Protocol or differently than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported. The Study Monitor is responsible for determining if all the INAD procedures are being followed as defined by the Study Protocol.

XX. PROTOCOL DEVIATIONS

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be contacted immediately for advice. Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation. Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with the quarterly data summaries and ultimately be submitted to the Study Director.

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Appendix IIIb
Sample of Knowledge Required for Position of USFWS Hatchery Manager (i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal and State agencies.

Knowledge of USFWS policy, programs, and organizational structure in order to be able to modify and adapt standard techniques/processes and to devise new strategies and plans necessary to overcome resource problems.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.